

CLAIMS

1/ Sequence of nucleotides coding for at least a part of the N-terminal region of a polypeptide specifically toxic towards larvae of the Lepidoptera of the family of the Noctuidae, and preferably towards S.littoralis, characterized by its capacity of hybridization with a gene capable of expressing a polypeptide toxic towards larvae of S.littoralis.

2/ Sequence of nucleotides of about 3 kb corresponding to the HindIII-PstI restriction fragment derived from B.thuringiensis capable of hybridizing with the probes 1, 2, 3 of pHTA2 reported in figure 2.

3/ Sequence according to Claim 1 or 2, characterized in that it contains in the following order the sites :

HindIII - HincII - BglIII - KpnI - HindIII - PstI.

4/ Sequence of nucleotides according to any one of the Claims 1 to 3, characterized in that it is obtained in vitro from a single strain of B.thuringiensis.

5/ Sequence of nucleotides according to Claim 4, characterized in that the strain of B.thuringiensis is the aizawai 7-29 strain.

6/ Sequence of nucleotides according to any one of the Claims 1 to 3, characterized in that it is obtained by in vitro genetic recombination of DNA sequences from two different strains of B.thuringiensis.

7/ Sequence according to Claim 6, characterized in that the 2 strains of B.thuringiensis correspond to the strains entomocidus 6-01 and aizawai 7-29, respectively.

8/ Sequence of nucleotides, characterized in that it codes for a polypeptide capable of forming an immunological complex with antibodies directed against polypeptides with a larvicidal activity towards S.littoralis.

9/ Sequence of nucleotides characterized in that it has the capacity to hybridize with a probe formed from the sequence (I) exhibiting the following chain arrangement :

10/ Sequence of nucleotides coding for a polypeptide toxic specifically towards larvae of the Lepidoptera of the family of the Noctuidae, and preferably towards S.littoralis, characterized in that it includes the chain arrangement (I) or (III) defined in Claim 9.

5 11/ Sequence of nucleotides according to Claim 9 or 10, characterized in that it has an ATG initiation codon situated at position 241.

12/ Sequence according to any one of the Claims 9 to 11, characterized by a GGAGG binding site to ribosomes at positions 230
10 to 234.

13/ Sequence according to one of the Claims 10 to 12, characterized in that it contains the sequence included between the nucleotides at position 137 and 177 (position -103 to -63) upstream from the ATG initiation codon) which is homologous to the extent of
15 about at least 70% with the region present upstream from the gene for the crystal of the strain kurstaki-HD1 Dipel (BTK) which contains the three promoters BtI, BtII and Ec, functional in B.thuringiensis and E.coli, respectively.

14/ Sequence of nucleotides, characterized in that it codes
20 for a polypeptide comprising the sequence of amino acids (II) below :

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15/ Recombinant expression and cloning vector containing at least a part of the nucleotide sequence such as defined in any one of the Claims 1 to 14.

16/ Plasmid according to Claim 15 characterized in that it is
5 pHT671 as represented in figure 4, or pHT71 comprising a HindIII-PstI DNA fragment constituted uniquely of DNA derived from the aizawai 7-29 strain.

17/ Modified bacterial strains, characterized in that after transformation they contain a sequence of nucleotides according to
10 one of the Claims 1 to 14.

18/ Bacterial strain according to Claim 17, characterized in that it contains at least one recombinant vector according to Claim 15 or 16.

19/ Polypeptide toxic towards larvae of the Lepidoptera and
15 preferentially towards S.littoralis, characterized in that it is capable of forming an immunological complex with antibodies directed against polypeptides with larvicidal activity towards S.littoralis.

20/ Polypeptide according to Claim 19, characterized in that it contains the sequence (II) or the sequence (IV) of amino acids
20 defined in Claim 14.

21/ Procedure for obtaining a nucleotide sequence coding for at least a part of the N-terminal region of a polypeptide toxic specifically towards larvae of the Lepidoptera of the family of the Noctuidae, and preferentially towards S.littoralis, characterized
25 by the following steps :

- the carrying out of a hybridization between a sequence of nucleotides from a strain of B.thuringiensis active against S.littoralis, on the one hand, and, on the other, one or several sequences of nucleotides utilized as probes derived from the 5' part of a restriction fragment
30 of a gene for a δ -endotoxin of B.thuringiensis, this part coding for the N-terminal part of a polypeptide toxic towards the Lepidoptera, and derived from the 3' part of this fragment coding for the COOH part of the polypeptide,
- the isolation of the fragment,
- 35 - its cloning in a vector, followed by its purification.

22/ Procedure according to Claim 21, characterized in that the hybridization probes utilized are obtained from a gene for a δ -endo-toxin derived from a aizawai 7-29 strain coding for a protein of 130kDa active against P.brassicae and inactive towards S.littoralis, this gene having been cloned in the recombinant plasmid pHTA2.

23/ Procedure according to Claim 21 or 22, characterized in that the fragment recombined with the vector in the cloning step is elaborated from at least one sequence of nucleotides derived from at least one recombinant vector containing a sequence of nucleotides from at least one strain of B.thuringiensis.

24/ Procedure according to Claim 23, characterized in that the fragment recombined with the vector in the cloning step is elaborated from several sequences of nucleotides derived from recombinant vectors containing sequences of nucleotides from at least 2 different strains of B.thuringiensis, possessing the same restriction maps and themselves containing all or part of the sequences of nucleotides capable of coding for a polypeptide active preferentially towards S.littoralis.

25/ Procedure according to Claim 23, characterized in that the fragment recombined with the vector in the cloning step is elaborated from a HindIII-PstI restriction fragment derived from the aizawai 7-29 strain.

26/ Procedure according to Claim 24, characterized in that the fragment recombined with the vector in the cloning step is elaborated from a HindIII-HincII restriction fragment derived from the entomocidus 6-01 strain and from a HincII-PstI restriction fragment derived from the aizawai 7-29 strain.

27/ Procedure according to Claim 22, characterized in that the restriction fragment recombined according to Claim 25 is carried preferentially by a plasmid pHTA6 and the restriction fragments recombined according to Claim 26, HindIII-HincII and HincII-PstI, are carried preferentially by the respective recombinant plasmids pHTE6 and pHTA6, the said plasmids pHTA6 and pHTE6 being those isolated with the aid of a probe constituted by a PvuII fragment of 2 kb of the plasmid pBT15-88 corresponding to the internal part of a gene for the chromosomal crystal of the berliner 1715 strain, from

transforming clones containing nucleotide sequences derived from B.thuringiensis strains active towards larvae of the Lepidoptera, inter-alia S.littoralis.

28/ Larvicidal composition with preferential activity towards
5 S.littoralis, characterized in that it contains an efficacious amount of polypeptide such as defined in any one of the Claims 19 to 20 expressed by the nucleotide sequences according to any one of the Claims 1 to 14, the vector according to the Claim 15, or the plasmid according to the Claim 16, or the bacterial strain according to any
10 one of the Claims 17 or 18.

29/ Application of the nucleotide sequences according to any one of the Claims 1 to 14 to produce a polypeptide toxic towards Lepidoptera, and preferentially S.littoralis, in microorganisms capable of expressing recombinant vectors containing these sequences such
15 as E.coli, B.subtilis, B.cereus or B.thuringiensis.

30/ Application according to Claim 29, characterized in that the sequences of nucleotides are introduced into microorganisms living in the environment or in association with the plants such as Pseudomonas, Azospirillum or Rhizobium and capable of expressing
20 recombinant vectors containing these sequences.

31/ Application according to Claim 29 or 30, characterized in that the nucleotide sequences are introduced into microorganisms in combination with different δ -endotoxin genes.

32/ Application of the nucleotide sequences according to any
25 one of the Claims 1 to 14 to the transformation of plants sensitive to S.littoralis, characterized in that it comprises the transfer and the expression of these sequences in these plants.

33/ Plant cells, the genome of which, after transformation by means of a non-essentially biological procedure, possesses in a stable
30 manner a sequence of nucleotides capable of expressing a polypeptide toxic towards S.littoralis, such as defined in any one of the Claims 1 to 14 and cells derived from their division.

34/ Plants having in particular S.littoralis as predator, transformed by a non-essentially biological procedure, the genome
35 of which possesses in a stable manner a sequence of nucleotides such

as defined in any one of the Claims 1 to 14, capable of expressing a polypeptide toxic towards S.littoralis and plants derived from their reproduction, their multiplication, or hybrid crosses.

35/ Plant having in particular S.littoralis as predator,
5 possessing in addition to their initial phenotypic and genotypic characters the property of expressing a polypeptide toxic preferentially towards S.littoralis, this property resulting from the insertion in its genome by genetic manipulation of a sequence of nucleotides capable of expressing the said polypeptide.

10 36/ Seed capable of giving rise to a plant according to Claim 34 or 35 or derived from such a plant, characterized in that it has integrated into its genome, by genetic manipulation, a sequence of nucleotides according to any one of the Claims 1 to 14.

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